Optimization of Condition on the High Speed and Efficient Methane Fermentation Process Pretreated Sub-Critical Water Hydrolysis Technology at Low Temperature Condition

Hayato Tokumoto, Masahiro Tanaka, and Hiroyuki Yoshida

Abstract: Novel biomass-energy process for production of methane from organic matter using sub-critical water (Sub-CW) hydrolysis as pretreatment was proposed. The model organic matters were used collagen as insoluble organics and BSA (Bovine Serum Albumin) as soluble organics. Methane was significantly produced by Sub-CW hydrolysis as pretreatment in the case of collagen. Methane production from collagen pretreated by Sub-CW at 423 K was increased about 6 times that from non-treated collagen. In acidogenic step, carbon dioxide production from collagen was increased about 40 times that from non-treated collagen. Furthermore, organic acids production from collagen was increased about 40 times that from non-treated collagen. In addition, methane fermentation was carried out using BSA as model of soluble organic matter. In methane production, there was little difference between non-treated and Sub-CW pretreatment using BSA. This was because BSA was soluble protein and had no advantage to hydrolyze. These results show that high speed and efficient methane fermentation process is attained using Sub-CW hydrolysis reaction as pretreatment at low temperature condition in the case of insoluble organic matters and Sub-CW pretreatment could be short cut the hydrolysis step and convenience for cooperative with acidogen and methanogen. In summary, Sub-CW technology at low temperature had a great effect on collagen such as insoluble organic matter.

Keywords: Sub-Critical Water, High Speed and Efficient Methane Fermentation, Organic Waste

1. INTRODUCTION
Currently, enormous organic matters (wet weight) per year are digested in dedicated industrial plants worldwide [1]. Methane fermentation is one of the main processes used for stabilization of organic matters. However, the conversion of organic wastes into methane in anaerobic fermentation process takes 1-2 months and more than 50% of non-digested organic wastes are remained after this particular step [2]. In anaerobic degradation of particulate organic material, particulate biopolymers such as proteins, carbohydrates and lipids are first hydrolyzed to organic monomers which can be used either as substrates by fermentative organisms (amino acids, sugar) or by anaerobic oxidizers (fatty acids). In the case of conventional process of methane fermentation by microorganism, rate-controlling step is degradation of organic matters. Yoshida et al. [5-8] has found that organic matters were decomposed into organic and amino acids in few minutes by Sub-CW hydrolysis reaction. Although decomposition of organic matters to CO₂ by supercritical water oxidation (SCWO) has attracted researchers’ attention for many years [3], Yoshida et al. [5-8] have shown that Sub-CW hydrolysis is more suitable for organic matters treatment than SCWO because various organic chemicals can be produced in short time (5-10 min) without addition of any chemicals. However, organic matters pretreated by Sub-CW at high temperature produce organic materials difficult to convert to acidogens and methanogens. By their productions and input energy are reduced using Sub-CW at low temperature, methane fermentation process using Sub-CW are made more efficient. In this study, high speed and efficient methane fermentation process using 393-513 K Sub-CW technology as pretreatment was investigated with collagen as model of insoluble organic matters and BSA as model of soluble organic matters.

2. MATERIALS AND METHOD
2.1. Sub-CW hydrolysis reaction
The details on experimental procedures were described by [8]. A stainless tube (SUS 316, 0.0168 m i.d. × 0.15 m length) with Swedgelok caps was used as a reactor (volume: 8.0×10⁻⁶ m³) for Sub-CW experiments. Dissolved oxygen in the sample was purged by blowing argon gas into the reactor. The reactor was then sealed by Swedgelok caps and immersed in a preheated molten salt bath (Thomas Kagaku Co. Ltd.) at 393-513 K for 5 min.

2.2. Methanogenesis and acidogenesis
All fermentation experiments were performed in auto sampler vial (PerkinElmer 20-CV) with butyl rubber and aluminum seal in batch mode at 310 K. Fixed volumes of inocula (5 ml) from a digester (operating at Yagi bio-ecology center, Yagi-cho, Kyoto) were supplemented with 1 ml of substrate under an atmosphere of N₂-CO₂ (80/20, vol/vol). Anaerobic sludge was fractionated into two fractions; a supernatant and a pellet, at 3000rpm for 10 min. Methanogens were localized on the pellet fraction [4, 9]. Therefore, in acidogenesis experiment, this supernatant (not exist methanogen) was used as the inoculums. Data are means ±SE of results from three independent samples.
2.3. Gas analysis

During the methane fermentation reaction, CH₄ and CO₂ concentrations were determined by removing 0.5 ml samples from the headspace of the vials with a gas-tight syringe. They were analyzed by gas chromatography (Shimadzu GC-8APT), equipped with a 3 m by 3.0 mm stainless steel 80/100 mesh Porapak Q column using thermal conductivity detector. Argon was used as a carrier gas at rate of 20 ml min⁻¹. The methane conversion was calculated with carbon balance.

2.4. Analysis of organic acids

The concentrations of the organic acids were measured using HPLC system (JASCO Gulliver series) incorporated with a combination of an ion exclusion column (Shedex KC-811) and post labeling equipped with spectrophotometer according to the standard method. The retention time of each organic acid was confirmed by injecting a sample in which a known amount of the authentic acid was added.

2.5. Carbon, hydrogen, nitrogen and sulfur contents of model substrate

Carbon, hydrogen, nitrogen and sulfur contents of model organic matters were analyzed with CHNS/O elemental analyzer (PerkinElmer 2400 Series II). The CHNS/O elemental analyzer oxidizes organic samples and quantifies generated gaseous components. Samples were dried in an oven (343 K) prior to the analysis. A few milligrams of the sample were loaded on the CHNS elemental analyzer.

3. RESULTS AND DISCUSSION

3.1. Methane production from collagen on Sub-CW pretreatment

Methane production from collagen pretreated by Sub-CW at different reaction temperatures (393-513K) for 5 minutes is shown in Figure 1. Methane productions by using Sub-CW pretreatment were 2.90-6.12 ml at 393-513 K in 10 days incubation. Conversely, methane productions of non-pretreated were 0.96 ml in 10 days incubation. Methane was most produced from collagen pretreated by Sub-CW reaction at 423 K. Methane production from collagen pretreated by Sub-CW at 423 K was increased is more than 6 times that from non-pretreated collagen. Yoshida et al. [5-8] has found that organic materials were degraded in few minutes by Sub-CW hydrolysis reaction at more than 473 K. However, methane was significantly produced that from non-pretreated collagen by low temperature condition of Sub-CW hydrolysis as pretreatment.

Table 1 shows methane conversion efficiencies of collagen in methane fermentation. Methane conversion efficiencies were analyzed by carbon balance which calculated carbon content of methane per carbon content of collagen. Methane conversion efficiencies by using Sub-CW pretreatment were 50.4-70.9% at 423 K in 4-10 days incubation. On the other hand, methane conversion efficiencies of non-pretreated were 6.6-11.2% in 4-10 days incubation. Methane conversion efficiencies were most efficient from collagen pretreated by Sub-CW reaction at 423 K.

Biogases were composed of methane and carbon dioxide in methane fermentation. Table 2 shows carbon dioxide conversion efficiencies of collagen in methane fermentation. Carbon dioxide conversion efficiencies by using Sub-CW pretreatment at 423 K were 21.6-27.3% in 4-10 days incubation. On the other hand, carbon dioxide conversion efficiencies of non-pretreated were 5.1-6.1% in 4-10 days incubation. Therefore, biogases (methane and carbon dioxide produced in methane fermentation) conversion efficiencies were 72.0-98.2% at 423 K in 4-10 days incubation (Table 1 and 2).

Table 1 Methane conversion efficiencies by methanogen

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Condition of Sub-critical water treatment</th>
<th>Methane conversion efficiencies [%] (Carbon content of methane / carbon content of collagen)</th>
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<tbody>
<tr>
<td>4 days</td>
<td>Non-treated</td>
<td>6.6</td>
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<tr>
<td></td>
<td>393 K</td>
<td>27.2</td>
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<td></td>
<td>423 K</td>
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<td>453 K</td>
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<td></td>
<td>483 K</td>
<td>30.5</td>
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<td>513 K</td>
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<tr>
<td>10 days</td>
<td>Non-treated</td>
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<tr>
<td></td>
<td>393 K</td>
<td>33.7</td>
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<td></td>
<td>423 K</td>
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<td>453 K</td>
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<td>483 K</td>
<td>45.8</td>
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<td>513 K</td>
<td>36.9</td>
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These result suggested that high speed and efficient methane fermentation process is attained using Sub-CW hydrolysis as pretreatment at low temperature condition.

3.2. Carbon dioxide production from collagen on Sub-CW pretreatment

Table 2 Carbon dioxide conversion efficiencies by acidogen and methanogen

<table>
<thead>
<tr>
<th>Incubation time</th>
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<th>Carbon dioxide conversion efficiencies [%] (Carbon content of carbon dioxide / carbon content of collagen)</th>
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<tr>
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<td>483 K</td>
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<td>513 K</td>
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These result suggested that high speed and efficient methane fermentation process is attained using Sub-CW hydrolysis as pretreatment at low temperature condition.

Fig. 1 Methane production from collagen by methanogen
To investigate the effect of Sub-CW pretreatment, carbon dioxide production from collagen which pretreated by Sub-CW at different reaction temperatures (393-513 K) for 5 minutes in the pre methane fermentation step such as acid fermentation is shown in Figure 2. In this experiment, the supernatant (without methanogen) of anaerobic sludge was used as the inoculums. Carbon dioxide productions by using Sub-CW pretreatment were 0.65-1.26 ml at 393-513 K in 8 days incubation. Conversely, carbon dioxide productions from non-pretreated were 0.26 ml in 8 days incubation. Carbon dioxide was most produced from collagen pretreated by Sub-CW reaction at 423 K.

Carbon dioxide production from collagen which pretreated Sub-CW at 423 K was increased about 5 times that from non-pretreated collagen.

Table 3 shows carbon dioxide conversion efficiencies of collagen in acid fermentation. Carbon dioxide conversion efficiencies were analyzed by carbon balance which calculated carbon content of carbon dioxide per carbon content of collagen. Carbon dioxide conversion efficiencies by using Sub-CW pretreatment were 8.9-14.1% at 323-513 K in 4 days incubation. On the other hand, carbon dioxide conversion efficiencies of non-pretreated were 2.9% in 4 days incubation. Carbon dioxide conversion efficiencies were most efficient from collagen pretreated by Sub-CW reaction at 423 K.

These results show that from non-treated and then formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, and valeric acid were produced by acidogen using Sub-CW pretreatment at 423 K. In addition, Sub-CW hydrolysis was produced 0.3-5 mM at 393-513 K such as total organic acid at low temperature condition (data not shown). Therefore, the production of organic acids in acid fermentation reactor was increased about 10 times using Sub-CW pretreatment at low temperature condition (Fig. 3).

Carbon dioxide conversion efficiencies were 71.4% at 423 K in 4 days incubation. On the other hand, carbon dioxide conversion efficiencies of non-pretreated were 1.8% in 4 days incubation. Organic acid conversion efficiencies were most efficient from collagen pretreated by Sub-CW reaction at 423 K.

These results show that Sub-CW pretreatment could be short cut the hydrolysis step and then convenience for bio-production was composed carbon dioxide and organic acid into acid fermentation. Figure 3 shows the concentration of organic acids in the acid fermentation reactor pretreated by Sub-CW hydrolysis at 423 K in 4 days incubation. In acidogenic step, total organic acids in acid fermentation reactor were generated about 40 times that from non-treated and BSA as model of soluble organic matters (Fig. 4). In methane production, there was little difference between non-treated and Sub-CW treatment. This was because BSA was soluble protein and had no advantage to hydrolyze.

These results show that Sub-CW pretreatment could be short cut the hydrolysis step and then convenience for...
cooperative with acidogen and methanogen. Therefore, high speed and efficient methane fermentation process is attained using Sub-CW hydrolysis technology at low temperature condition in the case of insoluble protein.

4. CONCLUSION

Methane production was increased significantly by using Sub-CW hydrolysis as pretreatment. Methane production from collagen pretreated at 423 K was increased about 6 times methane production that from non-treated. In acidogenic step, carbon dioxide production was increased about five times and then organic acids production in the acid fermentation reactor were increased about 40 times using collagen pretreated at 423 K that from non-treated. Organic acids were composed acetic acid, formic acid and propionic acid, butylic acid, isovaleric acid, and valeric acid in acid fermentation reactor. These results showed that high speed and efficient methane fermentation using Sub-CW technology at low temperature condition as pretreatment was attained. In methane production, there was little difference between non-treated and Sub-CW pretreatment using BSA. This was because BSA was soluble protein and had no advantage to hydrolyze. Therefore, Sub-CW pretreatment could be short cut the hydrolysis step and then convenience for cooperative with acidogen and methanogen.

REFERENCES


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Fig. 4 Methane production from BSA by methanogen

![Methane production from BSA by methanogen](image-url)